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NORMAL POSTMORTEM CHANGES IN THE BROWN SHRIMP, *PENAEUS AZTECUS*[1]

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ABSTRACT

A study was carried out to determine the normal rates and patterns of gross and histologic postmortem changes in the brown shrimp (*Penaeus aztecus* Ives). Experimental shrimp were held at 10°, 20°, or 30°C in water-saturated air or in seawater at a salinity of 30‰. Observations were made at 0, 2, 4, 8, 12, 24, 48, and 72 h postmortem.

The first change observed grossly was the onset of a rigorlike stiffening of the abdominal musculature. This stiffening was noted at 2 h postmortem at 30°C, but disappeared by 12 h postmortem. The condition appeared later and persisted longer at the lower temperatures.

Histologically, the tubule epithelium of the hepatopancreas was the first tissue to show autolytic change. The autolysis in the remaining tissues examined occurred in the following order: foregut and midgut epithelium, heart, neurons and nerve fibers, antennal gland epithelium, gill epithelium, epidermis, muscle, and lastly connective and cuticular tissue elements. In all tissues the rate of autolysis was temperature-dependent.

Shrimp from the Gulf of Mexico represent one of the most valuable fishery products of the United States. Their popularity as a food item and for use as sportfishing bait in some coastal areas has resulted in recent studies aimed at developing methods of artificially culturing these animals. Despite the enormous value of shrimp as a seafood, little is known about their histology and the rates and patterns of postmortem change.

Postmortem biochemical changes in the muscle of brown shrimp (*Penaeus aztecus* Ives) were reported by Flick and Lovell (1972). They reported that the compounds ATP, ADP, AMP, IMP, and glycogen decreased with time postmortem, while inosine, hypoxanthene, and lactic acid increased. The latter compounds were suggested as being partly responsible for flavor deterioration of ice-stored shrimp. Tissue pH values increased from 7.4 to 8.2 after 10 days in ice-stored shrimp (0°C), and, according to these authors, even with advanced bacterial spoilage, increases in pH are usually observed in fish and shellfish. Shrimp tails remained tender and soft during the entire storage period of 10 days (at 0°C) and did not exhibit any of the characteristics commonly associated with rigor mortis (Flick and Lovell, 1972).

In the only histologic study of postmortem change in an invertebrate animal, Sparks and Pauley (1964) reported the normal postmortem changes in the oyster, *Crassostrea gigas*. The digestive tubules of the oyster underwent the most rapid autolytic change in dead oysters held at 14°-16°C, while the Leydig tissue, gut, stomach, mantle, gill and palps autolyzed somewhat less rapidly. The gonads were the most resistant of all oyster tissue to autolysis with ova and sperm appearing relatively normal even after all other tissues had undergone extensive autolysis.

There are certainly a number of factors which influence the rate of autolysis in a dead animal. Some of these factors include water temperature, dissolved oxygen concentration, pH, bacterial flora of the water and of the animal, and the physiological condition of the animal at the time of death. It has been demonstrated in man and other animals that postmortem changes occur in a regular and irreversible pattern and at a relatively constant rate from one individual to another when factors causing variation in the rate and pattern are considered (Sparks and Pauley, 1964). Differentiation of histological changes due to disease from those due to postmortem autolysis or poor fixation is possible once the normal rates and patterns

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of postmortem changes under various conditions are known.

The present study was undertaken to determine the normal rates and patterns of postmortem change in penaeid shrimp as an aid in distinguishing gross and histologic changes due to autolysis from changes due to disease.

MATERIALS AND METHODS

Juvenile brown shrimp averaging 50 mm in total length (tip of rostrum to tip of telson) were obtained live from a commercial bait dealer and were held in 500-liter fiberglass tanks for several days prior to being killed. Control shrimp were killed by immersion in fixative. The remaining shrimp were killed by placing the shrimp between wet towels in an enamel tray for 30 min. The shrimp were removed after 30 min and placed into 100-ml glass jars. Two groups at three temperatures (10°, 20°, and 30°C) were studied: one in air and the other in seawater. Shrimp held in air were introduced wet into test jars and the jars were sealed. Shrimp held in water were introduced into the test jars and enough Instant Ocean[3] artificial seawater (at 30 ‰ salinity) was added to fill the jars. Jars were held in wire baskets at midlevel in constant temperature baths.

Samples for antemortem examination were taken at 0 h while those for postmortem examination were taken at 2, 4, 8, 12, 24, 48, and 72 h. Four control shrimp were taken for study and two shrimp (one from seawater and one from air) were taken from the 10°, 20°, and 30°C baths at each of the remaining sampling times.

General appearance, color, odor, and condition of the hepatopancreas were noted at each sampling period. Tissues for microscopic examination were preserved in 10% buffered Formalin, prepared for microscopy with standard paraffin embedding and sectioning methods, and stained with hematoxylin and eosin.

RESULTS

Gross Observations

The first change observed was the onset of a rigorlike condition of the abdomen which appeared at about 2 h after death at 30°C and at 4 and 24 h at 20° and 10°C, respectively. The abdomen became flaccid at 12 and 48 h after death in shrimp held at 30° and 20°C, but at 10°C the abdomen remained rigid at 72 h after death (Table 1).

[3] Reference to trade names in this publication does not imply endorsement of commercial product.

TABLE 1.—Time of onset of a rigor-like stiffening of shrimp abdominal musculature at 10°, 20°, and 30°C in air and seawater.

H postmortem	Temperature (°C) 10°C Air	10°C Water	20°C Air	20°C Water	30°C Air	30°C Water
0	−	−	−	−	−	−
2	−	−	+	−	+	+
4	−	−	+	−	+	+
8	−	−	+	+	+	+
12	−	+	−	+	−	−
24	+	+	+	+	−	−
48	+	+	−	−	−	−
72	+	+	−	−	−	−

\+ = stiff
− = flaccid

Color change and the appearance of spoilage odor were first observed at 4 h after death at 30°C. The general appearance of the shrimp changed from the usual semitransparent to a whitish-opaque at about the same time the first trace of odor was detected (Tables 2 and 3). At 20° and 10°C the first color change and appearance of odor were noted at 12 h and 24 h, respectively. At all three temperatures the color of the shrimp changed from opaque to an orange-red and finally to red with some blackened areas (Table 2). The intensity of spoilage odor increased along with the color change (Tables 2 and 3).

Fluid leakage from the hepatopancreas was first observed at 4 h at 30°C and at about 8 and 12 h postmortem at 20° and 10°C. Enzymatic digestion of hepatopancreas and surrounding tissues was grossly evident at 12 h at 30°C as

TABLE 2.—Times of postmortem color change of whole shrimp at 10°, 20°, and 30°C in air and seawater.

H postmortem	Temperature (°C) 10°C Air	10°C Water	20°C Air	20°C Water	30°C Air	30°C Water
0	−	−	−	−	−	−
2	−	−	−	−	−	−
4	−	−	−	−	0	0
8	−	−	−	−	0	0
12	0	−	−	0	LR	LR
24	LR	0	LR	LR	Rb	Rb
48	LR	LR	R	Rb	Rb	R
72	Rb	R	Rb	R	Rb	Rb

− = normal
0 = opaque
LR = orange to light red
R = red
Rb = red with blackened edges of cuticle or blackened appendages

TABLE 3.—Time of appearance of postmortem spoilage odor in whole shrimp held at 10°, 20°, and 30°C in air and seawater.

H postmortem	Temperature (°C) 10°C Air	10°C Water	20°C Air	20°C Water	30°C Air	30°C Water
0	–	–	–	–	–	–
2	–	–	–	–	–	–
4	–	–	+	–	+	+
8	–	–	++	–	+	+
12	–	–	++	–	++	++
24	+	+	++	++	++	++
48	++	+	+++	+++	+++	+++
72	++	++	+++	+++	+++	+++

– = normal
+ = odor

indicated by a loosening of the junction of the cephalothorax and abdomen. By 48 h the junction was very loose and by 72 h the tissues of the junction appeared mostly liquified. At 10° and 20°C the same process was observed but at a proportionately slower rate.

Histological Observations

Since the same patterns of autolysis were seen in shrimp held at all three temperatures, the differences being a function of time (Table 4), only the histological results from the 30°C portion will be presented in the text. The only significant histological differences between groups held in air and water noted were the more rapid tissue decomposition due to increased bacterial action in animals held submerged in seawater.

Digestive Tract

According to Roberts (1966), the digestive tract in shrimp is composed of three divisions: (1) the foregut, which includes the mouth, esophagus, stomach, and associated glands; (2) the midgut and hepatopancreas; and (3) the hindgut. Of these organs the hepatopancreas, the foregut, and midgut were studied in detail. The hindgut was not studied.

Hepatopancreas

The glandular hepatopancreas is the first organ to undergo autolytic change (Figure 1a). This organ is a compound tubularacinar exocrine gland composed of tubules which end in blind sacs or acini. The tubules and acini are lined with a simple low to high columnar epithelium (Figure 1b). Autolysis of the epithelium of this

TABLE 4.—Rate of postmortem histologic change in shrimp held in air or seawater at three temperatures.

a. Hepatopancreas.

H postmortem	Temperature (°C) 10°C Air	10°C Water	20°C Air	20°C Water	30°C Air	30°C Water
0	0*	0	0	0	0	0
2	2.5*	2.5	2.5	2.5	3	3.5
4	4	4	4	4	4	4
8	4	3.5	4.5	5	4.5	4.5
12	3.5	4	—	—	5	5
24	5	4	5	5	5	5
48	5	5	5	5	5	5
72	5	5	5	5	5	5

b. Midgut epithelium.

H postmortem	Temperature (°C) 10°C Air	10°C Water	20°C Air	20°C Water	30°C Air	30°C Water
0	0*	0	0	0	0	0
2	1*	1	1	1	1-2	1
4	2	1	1-2	1-2	—	2
8	4	—	4-5	—	3	4
12	—	3	2	4	5	4
24	5	4	—	5	5	—
48	5	5	5	5	—	5
72	5	5	5	5	5	5

c. Abdominal muscle.

H postmortem	Temperature (°C) 10°C Air	10°C Water	20°C Air	20°C Water	30°C Air	30°C Water
0	0*	0	0	0	0	0
2	0	0	0	0	0	0
4	1	1	2	1	1.5	1
8	0	1	2	2	2	2
12	1	1	2.5	2.5	3	3
24	3	3	3.5	3	3.5	3.5
48	2.5	3	4	4	3.5	4.5
72	4	3.5	4.5	4	4.5	4

d. Epidermis.

H postmortem	Temperature (°C) 10°C Air	10°C Water	20°C Air	20°C Water	30°C Air	30°C Water
0	0*	0	0	0	0	0
1	0	0	0	0	0	0
2	0	0	0	0	0	0
4	0	1	—	—	2	2
8	1	1	—	2	3	3
12	2	2	3	3	4	4
24	3	3	3	3	4	—
48	3	3	4	4	5	5
72	5	5	5	5	5	5

— No observation made.
* Average assigned values from a scale of 0 to 5 denoting the general histological appearance of the tissue or organ.
0 = Normal histologic appearance, like the control, no postmortem change.
1 = Slight change, scattered pyknotic nuclei, slight staining differences.
2 = More advanced cellular change with increases in nuclear pyknosis, karyrhexis, karyolysis, some cytolysis; loss of normal appearance or structure of the tissue or organ.
3 = Further advanced change with no normal appearing areas.
4 = Advanced autolytic change, tissue or organ represented by cellular debris or by its fibrous or cuticular stroma.
5 = Complete autolysis, tissue or organ no longer demonstrable.

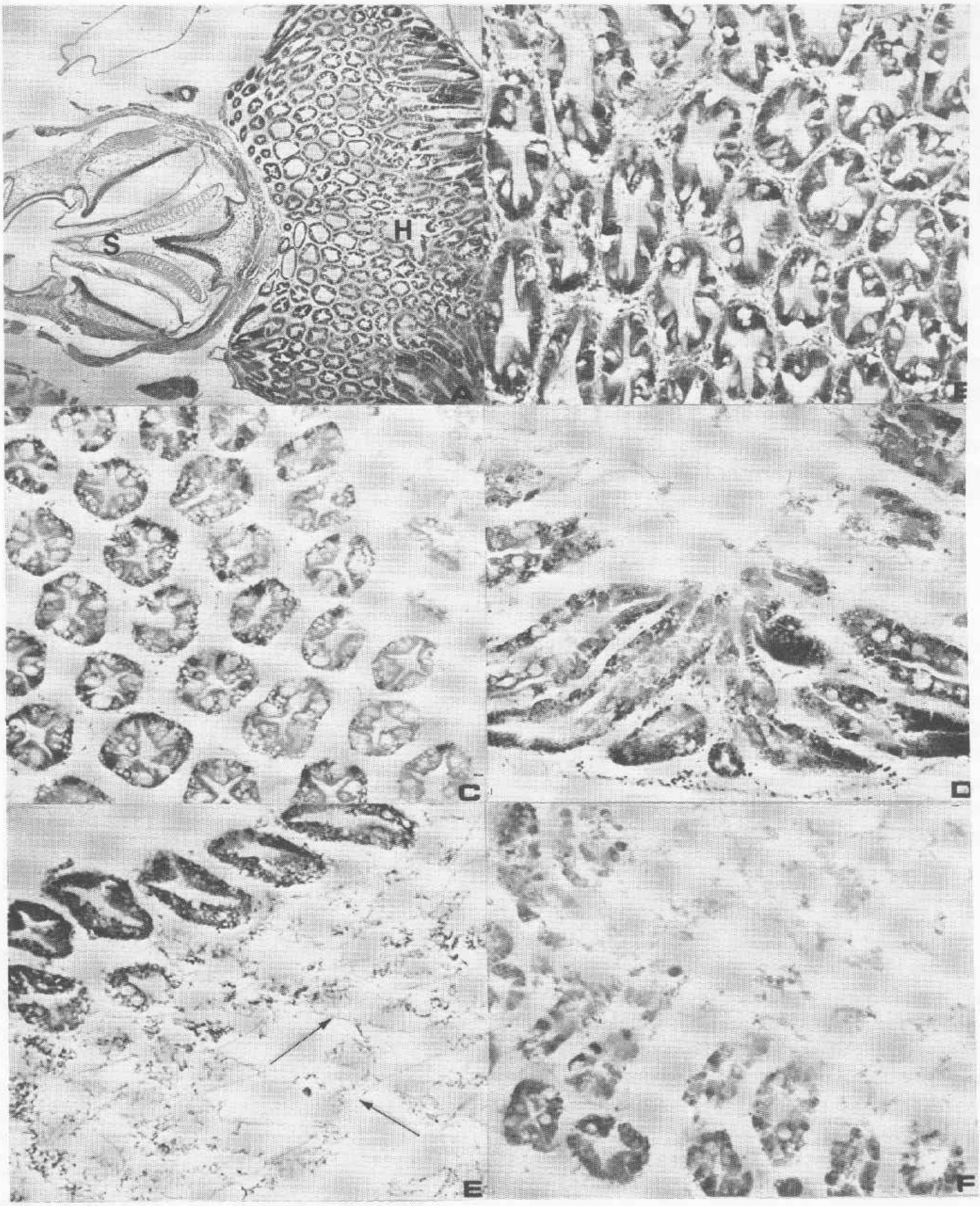

FIGURE 1.—**a.** Normal stomach (S) and hepatopancreas (H). 25×. **b.** Normal hepatopancreas. 120×. **c.** Hepatopancreas at 2 h postmortem showing edematous swelling between adjacent tubules. Autolysis is more advanced nearer the center of the organ (upper right) than at the periphery (left). 110×. **d.** Hepatopancreas at 4 h postmortem showing tubules on longitudinal section. Note the progression of autolytic change in the tubules from the periphery of the organ (bottom) to the autolyzed center (top). 110×. **e.** Hepatopancreas showing near complete autolysis (4 h postmortem). Note network of connective tissue stroma (arrows) remaining after autolysis of tubule epithelium. 120×. **f.** Hepatopancreas at 8 h postmortem showing advanced autolysis. Intensely pyknotic nuclei are present in the remaining epithelial cells near the periphery of the organ. Tissue debris and remnants of the connective tissue stroma are present nearer the organ's center (upper right). 120×.

organ proceeds so rapidly that by 2 to 4 h postmortem, the epithelium of tubules near the center of the organ showed advanced autolysis. These tubules showed desquamation and cytolysis of the lining epithelium and replacement with eosinophilic debris (Figures 1c and 1d). Nearer the periphery of the organ, the condition of the tubules and tubule epithelium appeared progressively more normal, with the most normal appearing tubules and acini at the periphery (Figures 1c and 1d). In the band of tissues between the normal appearing periphery and the lysed core, all stages of cell death were observed. A thin band of tissue in this area contained tubules whose epithelial cells possessed scattered pyknotic nuclei and had slight cytoplasmic staining differences (Figure 1e). Deeper to this layer the epithelial cells of tubules and acini possessed scattered pyknotic nuclei and had slight cytoplasmic staining differences (Figure 1e). The cytoplasm of these cells was highly vacuolated and stained variably with hematoxylin and eosin but generally much less basophilicily than normal (Figure 1c). At this time the spaces between adjacent tubules and acini had become swollen (Figures 1c and 1d). Slightly deeper to this layer epithelial cell nuclei had undergone karyorrhexis or karyolysis and disappeared. Many of the cells of this area had lysed and the cellular debris stained red with eosin. The supportive stroma of the hepatopancreatic tubules remained intact in some areas after the epithelium had autolyzed, thereby masking the former site of the hepatopancreatic tubules (Figure 1e).

By 8-12 h postmortem even the tubules and acini at the periphery of the organ showed advanced autolytic change, and the tissue debris and remnants of supportive stroma in the center of the organ were liquified (Figure 1f). The connective tissue capsule of the organ had become ruptured and few recognizable tubules were present. Past 12 h no trace of the hepatopancreas was present, and surrounding tissues had also been partially or completely digested, presumably by enzymes released from the autolyzed hepatopancreas.

Foregut and Midgut

Autolytic changes in the foregut, particularly the epithelium of the stomach (Figure 1a), proceeded at approximately the same rate as changes in the hepatopancreas. Nuclear changes within epithelial cells were observed at 2 h postmortem with considerable change by 4 h. By 8 to 12 h the epithelium of the stomach had undergone nearly complete autolysis and had disappeared, leaving only the cuticular elements of the stomach lining intact. The cuticular elements of the esophagus and stomach persisted for the duration of the study (72 h).

The midgut extends from the pyloric stomach to the sixth abdominal segment where it joins with the hindgut (Roberts, 1966). It is without a lining cuticle. The first autolytic change in the midgut was observed in the lining epithelium at 2 to 4 h, when the epithelial cells began to show changes such as scattered pyknotic nuclei, changes in staining reaction from a pale basophilic reaction to a more eosinophilic one, and the "blebing" of the apical ends of epithelial cells into the gut lumen (Figure 2a). The epithelium usually remained attached to the basement membrane at 2 h, but in some areas portions of the midgut epithelium had been sloughed into the gut lumen (Figures 2b and 2c). Sloughed epithelial cells were rounded and had intensely pyknotic nuclei and a uniform eosinophilic cytoplasm. At this time the gut lumen usually contained a fibrous, eosinophilic coagulum (Figure 2b). The gut wall basal to the lining epithelium showed no appreciable changes by 4 h.

By 8 to 12 h the midgut epithelium had been sloughed into the gut lumen (Figure 2d). The epithelial cells in the gut lumen were rounded, and some had pyknotic nuclei, but they were mostly anucleate. Many of the epithelial cells had lysed and left behind amorphous masses of eosinophilic debris (Figure 2d). Changes in the cellular elements of the wall of the midgut became apparent by 8-12 h. These changes consisted primarily of a decrease in nuclear number in the muscle and connective tissue cells present and pyknosis of those nuclei remaining (Figure 2d). In general, the cytoplasm of the cells present showed increased eosinophilia.

No trace of the lining epithelium was present after 24 h (Figure 2e). The coagulum, which was present in the gut lumen of some animals at 2-8 h, was still present. Also present in the gut lumen were large numbers of bacteria (Figure 2e). No nuclei were present in the gut wall, and the cellular elements remaining stained intensely with eosin.

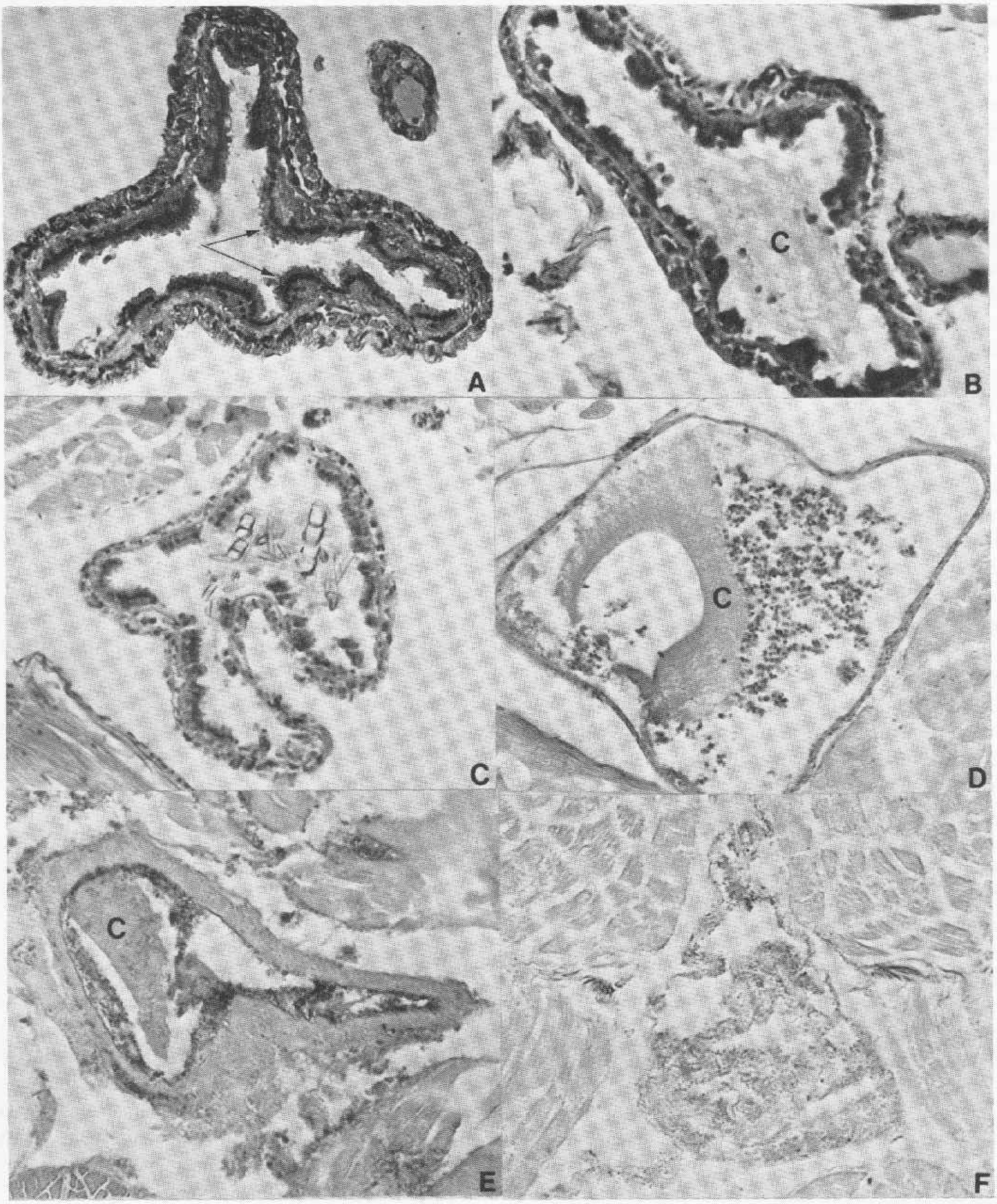

FIGURE 2.—**a.** Cross section of midgut at 2 h postmortem. The appearance is near normal except for the "blebing" of the apical ends of some of the epithelial cells (arrows) and a few pyknotic nuclei. 250×. **b.** Midgut showing more advanced autolytic change at 2 h postmortem. Some epithelial cells have been sloughed into the gut lumen where an eosinophilic coagulum (C) has formed. 240×. **c.** Midgut at 4 h postmortem. Most of the epithelial cells possess pyknotic nuclei, and some of the cells have been sloughed into the gut lumen. 210×. **d.** Midgut at 8 h postmortem. Sloughed epithelial cells are rounded and are either anucleate or have pyknotic nuclei. An eosinophilic coagulum is present. 160×. **e.** Midgut at 24 h postmortem. An eosinophilic coagulum is present in the gut lumen as are numerous bacteria. No trace of the gut epithelium remains. The muscle and fibrocyte cells of the gut wall are anucleate. 190×. **f.** Site of midgut at 48 h postmortem. Bacteria and debris have filled the gut lumen. Only fibrous elements of the gut wall remain. 150×.

By 48 h, the gut wall had become thin and was frequently interrupted. The gut lumen was filled with bacteria and other debris (Figure 2f). By 72 h, all traces of the gut, including the gut wall, had disappeared leaving the former site of the gut marked only by masses of bacteria and amorphous eosinophilic cellular debris.

Heart and Major Vessels

In shrimp the heart lies immediately dorsal and slightly caudad to the large hepatopancreas. Only the thin connective tissue coverings of the two organs separate them. Hence, autolysis of the hepatopancreas and release of its proteolytic enzymes results in a rapid destruction of the rather loose tissues of the shrimp heart (Figure 3a). The hepatopancreas showed considerable autolytic change by 4 h postmortem leaving the heart barely recognizable (Figure 3b). By 8 h the heart was not distinguishable from the other tissue debris present at the heart's former location in the cephalothorax. Vessels in the vicinity of the hepatopancreas and heart also disappeared by 4-8 h, but vessels elsewhere, such as in the abdomen, persisted much longer, some still recognizable after 24 h. However, by 48 h vessels were not usually demonstrable anywhere in the body of a shrimp.

Musculature

Shrimp locomotory muscle is striated and presents a histologic appearance that is similar to that of vertebrate striated muscle (Figure 4a). The muscles of the cephalothorax in the vicinity of the hepatopancreas underwent rapid autolytic change, apparently due to digestion by enzymes released on lysis of the hepatopancreas. Further from the hepatopancreas, the rate of autolytic change in the muscle was much slower. The earliest observed postmortem change in the muscle was at 4 h when some individual muscle fibers had a slightly "frayed" appearance. There was a pronounced swelling, presumably edematous, between adjacent muscle fibers (Figure 4b). By 8-12 h, muscle cell nuclei had become pyknotic. After 24 h muscle cells had become anucleate, highly eosinophilic, and the edematous swelling between adjacent muscle cells had decreased. Cross striations within muscle fibers were especially evident (Figures 4d, 4e, and 4f).

In some, but not all, of the shrimp studied, bacterial growth was evident between muscle bundles, especially in the vicinity of the gut. The presence of large numbers of bacteria greatly increased the rate of tissue deterioration (Figure 4c), while muscle not heavily invaded by bacteria remained recognizable as muscle tissue at 72 h (Figure 4f).

Integument

The integument, consisting of epidermis and an overlying cuticle, underwent rapid degeneration in the area of the cephalothorax that surrounds the hepatopancreas, leaving only